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Seasonal Changes in *Trans*-Aconitate and Mineral Composition of Crested Wheatgrass in Relation to Grass Tetany

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Highlight: *Grass tetany (hypomagnesemia) frequently occurs from March through June in cattle grazing crested wheatgrass in western United States. High levels of trans-aconitate and/or citrate, K, K/(Ca + Mg) ratios and low Mg in the grass are implicated in the etiology of the disease. In the moist 1967 season, during periods of "flush" growth following warming trends, trans-aconitate and K increased while Ca and Mg decreased in crested wheatgrass. These characteristics may explain the incidence of grass tetany during periods of "flush" growth. During the dry 1968 season, these trends were not observed. Growth chamber studies confirmed some of the reasons for changes in crested wheatgrass composition observed in 1967 and 1968.*

Grass tetany (hypomagnesemia), a metabolic disorder of ruminants, frequently occurs from March through June on pastures of crested wheatgrass (*Agropyron desertorum*) and other grasses in the western United States (Grunes et al., 1970). In the last 10 years at least 470 known cases of tetany and many suspected cases were noted in northern Nevada, mainly in the extreme

northeastern part of the state (unpublished Nevada State Office, BLM Report). Most reported cases were on crested wheatgrass, but losses also happened on native grasses. Tetany occurs in northeastern Nevada in early spring or late winter when cows are fed grass hay. Hjerpe (1964) reports tetany to be a major problem in California, with losses estimated at 4,000 to 6,000 head during the winter of 1963-64 and sporadic losses reported annually. Losses also occur in Idaho and Utah. Tetany may be prevalent during the autumn months, especially on regrowth after late summer showers.

Induction of the disorder involves many factors. Among factors that promote tetany are low temperatures or a change from low to high temperatures

with rapid growth when moisture is adequate (Kemp and 't Hart, 1957). During dry years incidence of tetany is much lower. High rates of K and N fertilization and low forage Mg are also involved (Kemp, 1958, 1960). The Mg content of grass tetany-prone pastures is between 0.07% and 0.20% dry weight basis, with averages of 0.17% for the Netherlands (Kemp and 't Hart, 1957) and 0.145% for Scotland (Butler, 1963). The critical level is controlled by the relative availability of Mg to the animal. Ratios of K/Ca + Mg (expressed as meq/kg) greater than 2.2 have been related to the occurrence of tetany (Kemp and 't Hart, 1957).

More recently, *trans*-aconitate of 1% or more in grass has been associated with tetany (Burau and Stout, 1965; Stout et al., 1967). Also, the dietary intake of 1% citric acid, as sodium citrate, reduced the Mg concentration in the blood serum of ruminants (Burt and Thomas, 1961). Bohman et al. (1969) induced tetany in cows by giving citric or *trans*-aconitic acid and KCl as an oral drench. For a comprehensive review of grass tetany, the reader is referred to the article by Grunes et al. (1970). The objective of this study was to

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evaluate the effect of environmental temperatures on changes in *trans*-aconitate, Mg, Ca, and K in crested wheatgrass and relate such changes to the incidence of grass tetany on spring ranges in northern Nevada.

Methods

During April and May of 1967 and 1968, crested wheatgrass samples were collected from a well established, non-grazed seeding at Orovada in Northwestern Nevada. Losses from grass tetany had occurred in this area in previous years. Sampling began when the blades were about 5 cm long and continued until the soft dough stage. About 100 g of fresh plant material were collected weekly by taking a few blades from randomly selected plants. The grass was cut from 2.5 to 5 cm above the ground. Samples were frozen with solid CO₂ or liquid N₂, subsequently freeze-dried and ground to pass a 40-mesh screen.

Sodium, K, Ca and Mg were determined on nitric-perchloric acid digests of dried plant material by atomic absorption spectroscopy. Chlorides were extracted with 0.1N nitric acid and determined potentiometrically (Brown and Jackson, 1955). Total aconitate was determined by a modification of a polarographic procedure (Burau, 1969). Fifty ml of extracting solution (0.1 M Na₂HPO₄-0.1 M NH₄Cl, pH 9.5) was added to 0.5 g of plant material and shaken for 1 hour in the presence of approximately 1 g of gas-produced carbon black. After filtering, a 10-ml aliquot of the extract was combined with 10 ml of 0.5 N HCl and made to a volume of 50 ml with water. A 25-ml aliquot was then placed in a polarographic electrolysis vessel, "H" form, and de-aerated with nitrogen. The diffusion current at -0.9 volt was measured and compared with a standard curve to give the concentration of total aconitate in the unknown.

Air temperatures were recorded on a thermograph in a standard U. S. Weather Bureau shelter 15 cm above the ground surface. The temperatures are shown as 5-day means. Rainfall data were taken from the U. S. Weather Bureau Station at Orovada approximately 2 km from the plots.

Soil samples were taken at 15-cm increments to a depth of 45 cm, dried and ground to pass a 2-mm sieve. Each sample was extracted with neutral 1 M ammonium acetate (Richards, 1954); the concentration of Na, K, Ca and Mg was determined by atomic absorption spectrophotometry. The pH of the saturated soil paste was measured with a glass electrode; soluble salts were estimated by electrical conductivity of extracts of saturated soil pastes (Richards, 1954).

In order to investigate the relation-

ships among temperature, "flush" growth, and changes in elemental and *trans*-aconitate contents of crested wheatgrass, a growth chamber study was undertaken.

For growth chamber studies, eight crested wheatgrass seeds were planted in each of 42 black plastic pots, 12.7 cm diameter and 15 cm high. Each pot was filled with a potting mix consisting of six parts loam, one part expanded vermiculite, and one part peat moss. The soil was kept moist and left in the greenhouse about 12 weeks until the grass was 18 to 23 cm tall. These pots were then transferred to a temperature and light controlled growth chamber. Plants were left in the chamber with a light intensity of 21,500 lux and a 10-hour dark and 14-hour light period for 15 days. Temperature was programed for a minimum of 6° C about an hour before the light period started and for a peak of 27° C midway through the light period. Temperatures were programed to follow the regimen of a typical warm spring day at Orovada.

After the first 15-day period was over, the photoperiod was left the same, but light intensity was lowered to 10,750 lux. The temperature was programed to follow a typical cool, cloudy spring day at Orovada with a low of -1° C and a high of 7° C. This regimen continued for 18 days. On the 18th day the grass was returned to the high light, high temperature regimen. Sufficient water was added to maintain good plant growth. Samples were taken periodically during the cool and warm periods by removing a few blades of grass from each pot. The grass was analyzed as previously described.

When the sampling period was over, the grass was clipped to a height of 2.5 cm and sprayed for aphids. Regrowth was not good because the grass appeared deficient in P and N. Each pot was fertilized with 3 g of diammonium phosphate, equivalent to 502 kg/ha of N and 556 kg/ha of P. The fertilized grass was grown under the high light, cool temperature regimen until the plants were 18 to 23 cm tall. The regimen was then changed to reduced light and cool temperatures for 17 days and then to high light and warm temperatures for the rest of the sampling period (30 days). Periodic samples were taken during both the cool and

warm periods and analyzed.

Results

1967 and 1968 Field Study

Mean annual rainfall at Orovada is 29.15 cm with a mean of 10.1 cm in March, April, and May. However, rainfall during the spring of 1967 was 2.9 greater than normal for March, April, and May. Soil moisture was adequate, and plants showed no water stress during the 1967 sampling season. The spring of 1968 was much drier than normal, following a dry winter. Precipitation totaled only 5.4 cm during March, April, and May. The majority of the precipitation, 2.6 cm, occurred during the first 2 weeks of April. Grass showed water stress by the May 11, 1968, sampling.

Soils were derived from well-drained mixed alluvium, silty loam in texture, and were not strongly developed morphologically, although the 30- to 45-cm depth was calcareous. The pH was approximately neutral (6.8 to 7.4). Soluble salts were low in the surface 15 cm but increased slightly with depth. Considerable volcanic ash was also present. Cations in the 1 M ammonium acetate extracts and the cation exchange capacity are shown in Table 1. The 1968 samples were from a site adjacent to the 1967 site and illustrate some of the natural variability. Soil K and Mg appeared adequate for plant growth.

During the moist 1967 season the *trans*-aconitate and K levels of crested wheatgrass paralleled each other (Fig. 1). Changes in K correlated significantly with maximum temperature changes. *Trans*-aconitate almost correlated with temperature changes. When air temperatures increased in early May, K and *trans*-aconitate increased while Ca and Mg decreased. Using the *t*-distribution test (Snedecor, 1956) and ignoring the first two sampling dates when the blades were less than 5 cm, K and T. A. were significantly (1%) higher and Ca and Mg were significantly lower (1%). At the beginning of the season, Mg values were above the 0.14% to 0.17% Mg levels of the tetany-

Table 1. Ammonium acetate (1-molar) extractable cations, and cation changeable capacity (meq/100 g) Orovada, Nevada, 1967 and 1968.

Location	Depth (cm)	Na	K	Ca	Mg	Exc. cap.
Orovada, 1967	0-15	0.5	1.8	7.2	3.7	12.9
	15-30	2.7	1.3	8.9	3.3	14.5
	30-45	6.3	0.9	26.0	4.0	14.8
Orovada, 1968	0-15	1.8	1.8	9.3	3.0	14.8
	15-30	5.1	1.2	10.3	2.9	15.1
	30-45	12.5	0.6	22.8	2.5	14.7

prone pastures of the Netherlands and Scotland. Magnesium reached tetany-prone levels (0.09 to 0.12%) following the increase in temperature.

The ratio of $K/(Ca + Mg)$ was low until the temperature increased and then rapidly increased to tetany-prone levels. Following the increase in temperature, $K/(Ca + Mg)$ was significantly higher (1%) than before the temperature increase. Chloride levels were 0.75% at the beginning of the season, fell to 0.5%, and rose to 0.64% at the end of the season. Sodium was low, less than 0.09%, throughout the season.

The rapid increase in blade length is indicative of the period of "flush" growth following the general rise in air temperature about May 5.

The dry 1968 season produced plant composition patterns that were different from those of the moist 1967 season (Fig. 2). Excluding the first sampling date, K and *trans*-aconitate values tended to parallel each other until soil moisture was depleted during the first week in May. When the temperature increased, K and *trans*-aconitate increased; but when moisture stress developed, *trans*-aconitate decreased. About a week later K also decreased. No significant difference was found in K or *trans*-aconitate between the cool and warm periods. There was no significant difference in *trans*-aconitate between the dry and wet years, but K was significantly (1%) lower in the dry year.

Magnesium and Ca were significantly

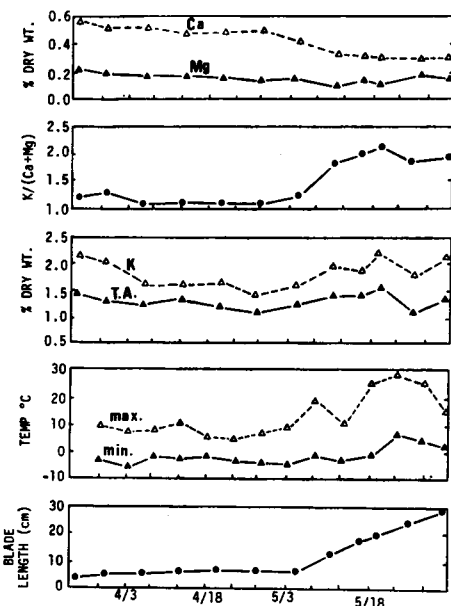


Fig. 1. Relation between air temperature (15 cm above soil surface), Ca, Mg, K, *trans*-aconitate (T. A.), $K/(Ca + Mg)$ (meq/kg) and blade length in crested wheatgrass at Orovala, Nev. Spring 1967.

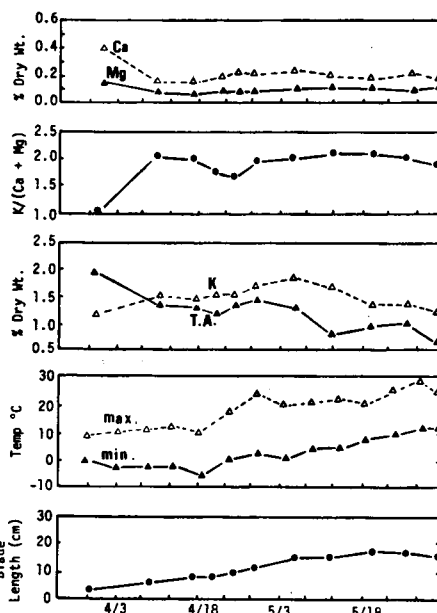


Fig. 2. Relation between air temperature (15 cm above soil surface), Ca, Mg, K, *trans*-aconitate (T. A.), $K/(Ca + Mg)$ (meq/kg) and blade length in crested wheatgrass at Orovala, Nev. Spring 1968.

(1%) lower during the dry 1968 season, but no difference was found between the cool and warm periods. The $K/(Ca + Mg)$ ratios reached a peak at the beginning of the season and declined slightly when the grass showed moisture stress. The ratios never reached more than 2.0, and there was no significant difference between the cool and warm period in the dry year. There was also no significant difference in maximum $K/(Ca + Mg)$ ratios in the wet (1967) and dry year (1968). Chlorides were slightly lower (approx. 0.5%) than in the wet year and remained essentially unchanged throughout the season. Sodium was also lower than in the previous season (less than 0.04%) and remained essentially constant.

Blade length did not increase as rapidly and was not as great following the temperature increase in 1968 as in 1967 because moisture stress symptoms had begun to develop. Crested wheatgrass did not show any sustained period of "flush" growth in 1968.

In the growth chamber experiments, Ca and Mg in both the fertilized and nonfertilized crested wheatgrass decreased about 6 to 10 days after temperature and light had been increased (Fig. 3). The difference in Ca and Mg between the cool and warm periods were significant (1%) for the nonfertilized crested wheatgrass. The differences were also significant at the 1% level for Ca and significant at the 5% level for Mg in the fertilized

crested wheatgrass. The Ca concentration of the fertilized grass dropped about 3 days sooner than in the nonfertilized grass. The change in Mg was not as great as in Ca, but Mg concentration of both the fertilized and nonfertilized grass was lowest about 14 days after temperature was increased.

As the cool period continued, K became progressively lower in both the fertilized and nonfertilized grass. About 6 days after temperature and light were increased, K increased rapidly in the fertilized grass and remained about the same in the unfertilized. In the fertilized grass, K was significantly higher (5%) in the warm period; in the nonfertilized grass, the reverse was found. *Trans*-aconitate concentration also increased about 6 days after temperature and light were increased. The differences in *trans*-aconitate between the cool and the warm periods were significant in the unfertilized grass but not in the fertilized grass. Potassium and *trans*-aconitate concentrations tended to parallel each other in fertilized grass.

The ratio of $K/(Ca + Mg)$ also increased between 6 to 10 days after temperature and light were increased in the fertilized and nonfertilized grass. The $K/(Ca + Mg)$ was significantly higher (nonfertilized 5% level, fertilized 1% level) in the warm period. In the fertilized grass, the ratio increased more rapidly and reached a high point of 4.0 in about 15 days. After the peak was reached, the ratio dropped to about 3.5 and stayed there for the remainder of the experiment.

Discussion

During 1967, outbreaks of grass tetany occurred the week of May 16 in northern Nevada (about 25 km north of the plots) and southern Idaho. This was the first week with minimum temperatures above 0° C. A few cases of tetany are normally reported each year on seedlings adjacent to the sample plots. In 1967, ranchers in this area delayed turn-out until the tetany season had passed and thus avoided the possibility of high death losses. No cases of tetany were reported during the dry spring of 1968 in the Orovala area. However, in the wetter parts of northern Nevada and southern Idaho, outbreaks of tetany were reported shortly after May 1, when temperatures started to rise.

In the moist spring of 1967, the tetany-prone period began about a week after the temperature changed from cool to warm. At this time some of the factors

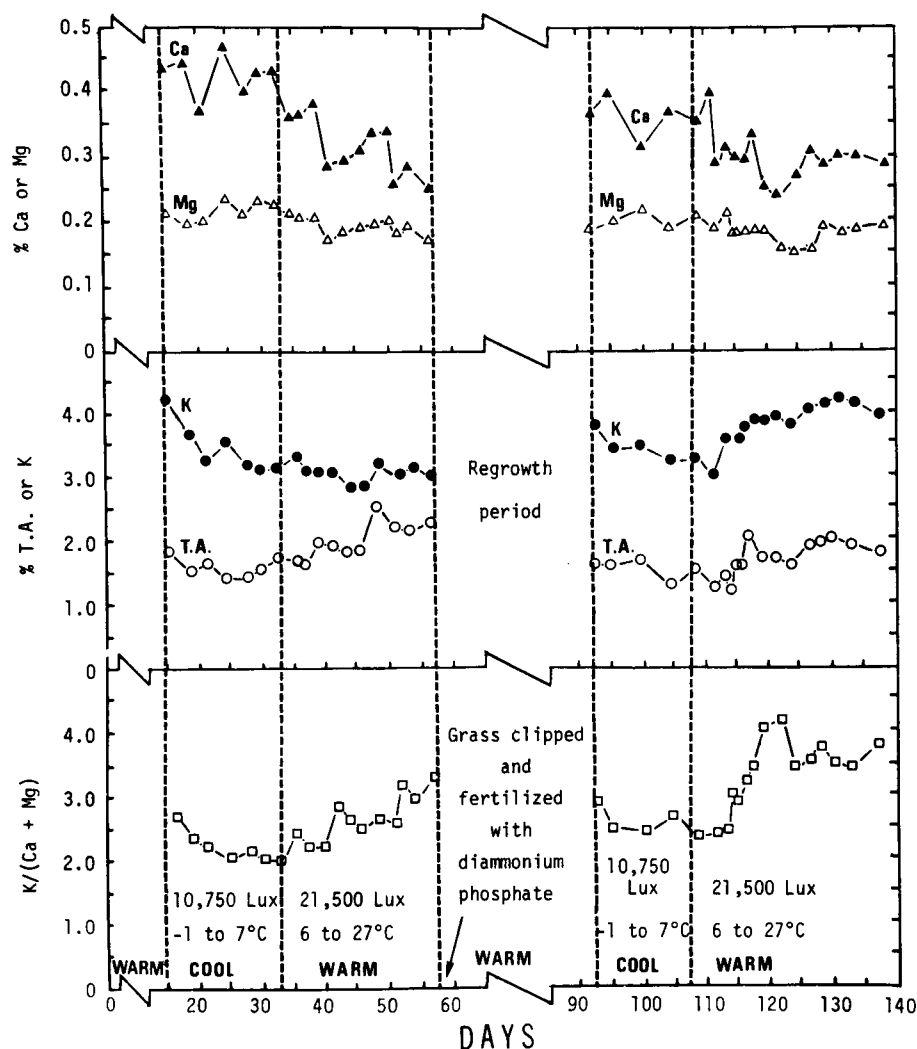


Fig. 3. Changes in Ca, Mg, K, trans-aconitate (T. A.), and $K/(Ca + Mg)$ (meq/kg) in crested wheatgrass grown in a growth chamber, when changed from a cool (-1°C to 7°C) low light (10,750 lux) regimen to a warm (6°C to 27°C), high light (21,500 lux) regimen. After the first cycle, grass was clipped and fertilized with diammonium phosphate.

probably results from optimization of tetany inciting factors, in addition to those explained by the changes in elemental content (Grunes et al., 1970).

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involved in the induction of grass tetany were optimal: high K and trans-aconitate levels, low Ca and Mg, and a rapid increase in the ratio of $K/(Ca + Mg)$. This occurred at the same time as a period of "flush" growth induced by ample moisture and rising temperatures.

In the dry spring of 1968, no period of "flush" growth was observed and no tetany was reported in the Orovalda area. Potassium and trans-aconitate declined as moisture became limiting, and Ca and Mg were low. The ratio of $K/(Ca + Mg)$ was consistently rather high as a result of low Ca and Mg values.

Soil contained ample Mg, but crested wheatgrass from the Orovalda site was low in Mg. Similar results were obtained for samples collected throughout northern Nevada (unpublished data of the authors). Apparently, crested wheatgrass and other grasses (unpublished data of the authors) are unable to accumulate concentrations of Mg required for good animal nutrition, even though the soils

appear to contain adequate Mg. In the greenhouse study, the potting mixture did not contain soil from the Orovalda area, and the Mg concentrations in the plants were appreciably higher.

The rapid increase in $K/(Ca + Mg)$ observed during 1967 when temperature was increased may be similar to the rapid increase in the ratio observed in fertilized growth chamber herbage upon warming (Fig. 3). Experience has shown that tetany proneness in the field is of several weeks duration, and this period may coincide with a period of rapid increase in the $K/(Ca + Mg)$ values. The initiation of tetany may result from this rapid change in elemental concentration, rather than a particular level. We hypothesized that tetany did not occur in 1968 because there was no rapid change in the ratio even though the ratio was high throughout the entire period. The prevalence of grass tetany in cattle grazing rapidly growing crested wheatgrass following a change from cool to warm temperatures